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(54) Fungal stress proteins.

(57) A polypeptide sequence from Candida albicans is described which has significant sequence homology with known stress proteins from other organisms, particularly the heat shock protein hsp 90 of Sacchromyces cerevisiae . Corresponding DNA sequences are also described, together with antibodies raised against fragments of the sequence. The polypeptide and DNA sequences and antibodies provide separate means for the diagnosis and/or treatment of fungal, particularly Candida, infections.

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FUNGAL STRESS PROTEINS

Field of the Invention

This invention relates to fungal stress proteins, to corresponding DNA sequences, to fungal stress protein inhibitors and to their use in medicine and for diagnosis.

Background to the Invention

Environmental stress can induce an increase in the rate of synthesis of so-called heat shock, or stress, proteins in both procaryotic and eucaryotic cells [see for example Schlesinger et al (eds) in Heat Shock from Bacteria to Man, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1972)]. Although the function of stress proteins has yet to be finally resolved, some have been reported to participate in assembly and structural stabilisation of cerain cellular and viral proteins, and their presence at high concentration may have an additional stabilising effect during exposure to adverse conditions.

Many pathogenic organisms have been shown to produce stress proteins [see for example Young D, et al , Proc. Natl. Acad. Sci. USA 85 , 4267-4270 (1988)]. The proteins are thought to be produced in response to the stress of infection to help protect the invading pathogen. Thus, for example, the ability to produce stress proteins has been implicated in the survival of bacterial pathogens within macrophages [Christmas, M.F. et al , Cell, 41 , 753-762 (1985) and Morgan, R.W. et al , Proc. Natl. Acad. Sci. USA, 83 , 8059-8063, (1986)].

It has been suggested that the presence of stress proteins in a variety of human pathogens indicates that the stress response is a general component of infections, and that stress proteins should be considered among candidates for subunit vaccines [Young, D. et al , ibid].

Candida albicans is, medically, the most important of the human fungal pathogens. Systemic candidiasis (candidosis) is an increasingly common cause of death amongst immunocompromised and debilitated patients, with a mortality of over 70% [Gold, J.W.M., Am. J. Med. 76 , 458-463, (1984)]; while oral candidiasis is a frequent early manifestation of the acquired immunodeficiency syndrome [Klein, R.S. et al , N. Engl. J. Med. 311 , 354-357, (1984)]. Candidiasis is difficult to diagnose, and is not easy to treat, mainly since the usual method of treatment involves use of amphotericin B, which is itself highly toxic. A need therefore exists in the diagnosis and treatment of Candida infections for more sensitive diagnostic methods and treatment which has less toxic side effects.

A number of Candida antigens have been detected in the sera of patients with systemic candidiasis [Matthews, R.C. et al, J. Clin. Microbiol. 25 , 230-237 (1987)]. One of these, with a relative molecular mass of approximately 47 kilodaltons (47kd) is an immunodominant antigen which has been reported by four independent groups [Matthews R.C., et al Lancet ii, 1415-1418 (1984); Au-Young, JK et al , Diagn. Microbiol. Infect. Dis., 3 , 419-432, (1985); Neale T.J., et al , Aust. N.Z.J. Med., 17 , 201-209 (1987); and Ferreira R.P. et al , J. Clin. Microbiol. 28 , 1075-1078 (1990)]. This 47Kd antigen is distinct from the 48-52Kd antigen described by Strockbine et al [Strockbine N.A. et al Infect. Immun. 43 , 715-721 (1984)] for two reasons:

(1) monoclonal antibodies raised against the 48-52Kd antigen cross-react with antigens at 120-135Kd and 35-38Kd [Strockbine N.A. et al , Infect Immun 43 , 1012-18 (1984); Buckley H.R., et al U.S. Patent No. 4670382 (1987)] and

(2) the 48-52Kd antigen it is an enolase [Safranek W.W. and Buckley H.R., Second Conference on Candida and Candidiasis, Abstract A7 (1990)]. In contrast, antibody to the 47Kd antigen cross-reacts with an antigen at about 92Kd (see below). An immundominant C. albicans antigen of 54.3Kd (range 48.9 to 59.7Kd) was described by Greenfield R.A., and Jones J.M., in Infect. Immun. 34 , 469-477 (1981).

Summary of the Invention

We have now been able to clone and express part of the DNA sequence encoding the 47Kd antigen, and in doing so we have surprisingly discovered a polypeptide sequence which has significant sequence homology with known stress proteins from other organisms, particularly the heat shock protein hsp 90 of Saccharomyces cerevisiae [Farrelly F.W. and Finkelstein D.B., J. Biol. Chem. 259 , 5745-5751 (1984)]. In Saccharomyces two genes exist, hsp 90 and hsc 90, coding for 98% homologous proteins [Finkelstein D.B.

and Farrelly F.W., Fed. Proc. 43 , 1499 Abstr. 482 (1984)]. Either of the genes can be deleted without affecting cell viability, however, deletion of both genes is lethal. The separate genes produce a constitutive and an inducible form [D. Finkelstein, personal communication cited in Lindquist S. Ann. Rev. Biochem. 55 , 1151-91 (1986)].

We can also now suggest that there are two forms of a C.albicans stress or heat shock protein hsp90. Thus a 47Kd breakdown product occurs on immunoblots of C.albicans grown at 37°C or 23°C whereas a 92Kd antigen only appears at 37°C. This suggests a heat-inducible, stable hsp 90 is produced at 37°C, but not at 23°C, whereas a more labile hsp 90 occurs at 23°C which breaks down to the 47Kd subcomponent under denaturing conditions. We have used this discovery to develop means for the improved diagnosis and treatment of candida infections and related fungal disease.

Thus according to one aspect of the invention we provide a fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1):

```
                10        20        30        40        50        60
               EFRAILFVPKRAPFDAFESKKKKNNIKLYVRRVFITDDAEELIPEWLSFIKGVVDSEDLP

                70        80        90       100       110       120
               LNLSREMLQQNKILKVIRKNIVKKMIETFNEISEDQEQFNQFYTAFSKNIKLGIHEDAQN

(1)            130       140       150       160       170       180
               RQSLAKLLRFYSTKSSEEMTSLSDYVTRMPEHQKNIYYITGESIKAVEKSPFLDALKAKN

               190       200       210       220       230       240
               FEVLFMVDPIDEYAMTQLKEFEDKKLVDITKDFELEESDEEKAAREKEIKEYEPLTKALK

               250       260       270       280       290       300
               DILGDQVEKVVVSYKLVDAPAAIRTGQFGWSANMERIMKAQALRDTTMSSYMSSKKTFEI

               310       320       330       340       350       360
               SPSSPIIKELKKKVETDGAEDKTVKDLTTLLFDTALLTSGFTLDEPSNFAHRINRLIALG

               370       380       390
               LNIDDDSEETAVEPEATTTASTDEPAGESAMEEVD*
```

or a fragment thereof, or an analogue thereof.

The single letters in formula (1) are each to be understood to represent a separate amino acid, and each is the conventional single letter symbol used for amino acids.

The stress protein according to the invention may be of fungal origin and may be obtainable, for example, from strains belonging to the genera Candida, for example Candida parapsilosis , Candida krusei and, in particular,

Candida albicans and Candida tropicalis ; Cryptococcus, for example Cryptococcus neoformans ; Histoplasma, for exampl Histoplasma capsulatum , and related yeasts; and Aspergillus, for example Aspergillus fumigatus and related filamentous fungi.

Particular fragments of a stress protein according to the invention include any peptide epitopes, for example of a few amino acids or analogues thereof. Examples of such epitopes include STDEPAGESA, LSREM, LKVIRK and LKVIRKNIVKKMIE. P ptides of this type may be synthesised using conventional liquid or solid phase peptide synthesis techniques.

Analogues of a stress protein according to the invention include those proteins wherein one or more amino acids in th sequence of formula (1) is replaced by another amino acid, providing that the overall functionality of the protein is conserved.

A stress protein according to the invention may be obtained in a purified form, and thus according to a further aspect of the invention we provide a substantially pure fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1), and analogues thereof.

The term substantially pure is intended to mean that the stress protein according to the invention is free from other proteins of fungal origin. In the various aspects of the invention described hereinafter it is to be understand that a reference to the fungal stress protein also includes substantially pure preparations of the protein.

In a further aspect the invention particularly provides a recombinant fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1) or a fragment thereof, or an analogue thereof.

A stress protein according to the invention may be further characterised by one or more of the following features:

(1) it has an isoelectric point (pI) in a range around pI 4 to pI 5;

(2) it is an immunodominant conserved antigen;

(3) patients recovering from systemic candidiasis seroconvert to the stress protein;

(4) patients with acquired immunodeficiency syndrome have antibody to the stress protein;

(5) it cross-reacts with the 47 kilodalton and approximately 92 kilodalton antigens of Candida albicans using affinity-purified polyclonal monospecific antibody against the 47 kilodalton antigen.

A stress protein according to the invention has a number of uses. Thus, for example, the protein may form the basis of a diagnositic test for fungal infection, for example an immunological test such as an enzyme-linked immunosorbent assay, a radioimmunoassay or a latex aggulutination assay, essentially to determine whether antibodies specific for the protein are present in an organism.

In another use, the stress protein according to the invention may be employed, using conventional techniques, for screening to obtain activity inhibiting agents for use in the treatment of fungal infections. Such a screening method forms a further aspect of the invention.

In a further use, the stress protein according to the invention is particularly well suited for the generation of antibodies. Thus according to a further aspect of the invention we provide a fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1) or a fragment thereof or an analogue thereof, for use as an immunogen.

Standard immunological techniques may be employed with the stress protein in order to use it as an immunogen. Thus, for, example, any suitable host may be injected with the protein and the serum collected to yield the desired polyclonal anti-stress protein antibody after purification and/or concentration. Prior to injection of the host the stress protein may be formulated in a suitable vehicle and thus according to a further aspect of the invention we provide a composition comprising a fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1) or an analogue thereof together with one or more pharmaceutically acceptable excipients.

For purification of any anti-stress protein antibody, use may be made of affinity chromatography employing an immobilised stress protein of the invention as the affinity medium. This according to another aspect of the invention we provide a fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1), or a part thereof or an analogue thereof, covalently bound to an insoluble support.

The use of the stress proteins according to the invention as immunogens for the production of antibodies generates one type of inhibitor of the action of the protein. Generally, inhibitors of the stress proteins are potentially useful in the diagnosis, and in particular the treatment, of fungal infections and provide a further feature of the invention. Inhibitors include any antagonists of the action of the stress proteins or agents which prevent their production, and in particular include those which may be used in the treatment of fungal infections. Suitable inhibitors include for example pharmaceutical reagents, including antibodies, and chemical analogues of the stress proteins to antagonise the action of the stress protein, and anti-sense RNA and DNA to prevent production of the stress protein. Suitable inhibitors may be determined using appropriate screens, for example by measuring the ability of a potential inhibitor to antagonise the action of, or prevent the production of a stress protein according to the invention or a fragment thereof, or an analogue thereof, in a test model for example an animal model such as the mouse model described in the Examples hereinafter.

According to a further aspect of the invention we provide an inhibitor of a fungal stress protein, said protein having an amino acid sequence which includes at least the sequence of formula (1) or a fragment

thereof or an analogue thereof, for use in the diagnosis or treatment of fungal infections.

Inhibitors may be used either alone or where appropriate in combination with other pharmaceutical agents, for exampl , other anti-fungal agents, such as amphotericin or flucytosine.

One particularly useful group of inhibitors according to this aspect of the invention are antibodies capable of recognising and binding to the stress proteins.

Thus, according to yet another aspect of the invention we provide an antibody specific for one or more epitopes of a fungal stress protein having an amino acid sequence which includes at least the sequence or formula (1) or a fragment thereof or an analogue thereof.

The antibody may be a whole antibody or an antigen binding fragment thereof and may in general belong to any immunoglobulin class. Thus, for example, it may be an immunoglobulin M antibody or, in particular, an immunoglobulin G antibody. The antibody or fragment may be of animal, for example mammalian origin and may be for example of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or, if desired, a recombinant antibody or antibody fragment, i.e. an antibody or antibody fragment which has been produced using recombinant DNA techniques.

Particular recombinant antibodies or antibody fragments include, (1) those having an antigen binding site at least part of which is derived from a different antibody, for example those in which the hypervariable or complementarity determining regions of one antibody have been grafted into the variable framework regions of a second, different antibody (as described in European Patent Specification No. 239400); (2) recombinant antibodies or fragments wherein non-Fv sequences have been substituted by non-Fv sequences from other, different antibodies (as described in European Patent Specifications Nos. 171496, 173494 and 194276); or (3) recombinant antibodies or fragments possessing substantially the structure of a natural immunoglobulin but wherein the hinge region has a different number of cysteine residues from that found in the natural immunoglobulin, or wherein one or more cysteine residues in a surface pocket of the recombinant antibody or fragment is in the place of another amino acid residue present in the natural immunoglobulin (as described in International Patent Applications Nos. PCT/GB 88/00730 and PCT/GB 88/00729 respectively).

The antibody or antibody fragment may be of polyclonal, or preferably, monoclonal origin. It may be specific for a number of epitopes associated with the stress protein but it is preferably specific for one.

Antigen binding antibody fragments include for example fragments derived by proteolytic cleavage of a whole antibody, such as $F(ab')_2$,Fab' or Fab fragments, or fragments obtained by recombinant DNA techniques, for example Fv fragments (as described in International Patent Application No. PCT/GB 88/00747).

The antibodies according to the invention may be prepared using well-known immunological techniques employing the stress protein as antigen. Thus, for example, any suitable host mat be injected with the stress protein and the serum collected to yield the desired polyclonal antibody after appropriate purification and/or concentration (for example by affinity chromatography using the immobilised stress protein as the affinity medium). Alternatively splenocytes or lymphocytes may be recovered from the stress protein injected host and immortalised using for example the method of Kohler et al , Eur. J. Immunol. 6 , 511, (1976), the resulting cells being segregated to obtain a single genetic line producing monoclonal anti-fungal stress proteins. Antibody fragments may be produced using conventional techniques, for example by enzymatic digestion, e.g. with pepsin [Parham, J. Immunol, 131 , 2895 (1983)] or papain [Lamoyi and Nisonoff, J. Immunol. Meth., 56 , 235, (1983)]. Where it is desired to produce recombinant antibodies according to the invention these may be produced using for example the methods described in European Patent Specifications Nos. 171496, 173494, 194276 and 239400.

Antibodies according to the invention may be labelled with a detectable label or may be conjugated with effector molecule for example a drug e.g. an anti-fungal agent such as amphotericin B or flucytosine or a toxin, such as ricin, or an enzyme, using conventional procedures and the invention extends to such labelled antibodies or antibody conjugates.

The antibodies according to the invention have a diagnostic and/or therapeutic use. Thus for diagnostic use the antibodies may be employed to detect whether the stress protein is present in a host organism, to confirm whether the host has a particular fungal infection, for example an infection due to a Candida, Cryptococcus, Histoplasma or Aspergillus organism, for example in the diagnosis of fungal abcesses, especially hepatic Candidiasis, and/or to monitor the progress of therapeutic treatment of such infections. Diagnostic methods of this type form a further aspect of the invention and may generally employ standard techniques, for example immunological methods such as enzyme-linked immunosorbent methods, radioimmuno-methods, lates agglutination methods or immunoblotting methods.

Antibodies according to the invention also have a therapeutic use in the treatment of fungal infections, for xample those just described and may be used alone or conjugated to an effector molecule, in the latter

case to target the effector molecule, .g. an anti-fungal agent such as amphotericin B or flacytosine, to th infecting organism. For therapeutic use the antibody may be formulated in accordance with conventional procedures, for example with a pharmaceutically acceptable carrier or excipient, e.g. isotonic saline for administration at an appropriate dosage, depending on the nature of the infection to be treated and the age and condition of the patient.

If desired, mixtures of antibodies may be used for diagnosis or treatment, for example mixtures of two or more antibodies recognising different epitopes of a fungal stress protein according to the invention, and/or mixtures of antibodies of a different class, e.g. mixtures of IgG and IgM antibodies recognising the same or different epitope(s) of a fungal stress protein of the invention.

The stress proteins according to the invention may be prepared by a variety of processes, for example by protein fractionation from appropriate fungal cell extracts, using conventional separation techniques such as ion exchange and gel chromatography and electrophoresis, or by the use of recombinant DNA techniques, as more particularly described in the Examples hereinafter. The use of recombinant DNA techniques is particularly suitable for preparing substantially pure stress proteins according to the invention.

Thus according to a further aspect of the invention we provide a process for the production of a fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1) or an analogue thereof, comprising the steps of (1) culturing a host organism transformed with a vector including a gene coding for a precursor of said protein, (2) cleaving said precursor to produce said protein and (3) recovering said protein.

Preferably the precursor cleaved in this aspect of the invention is a fusion protein comprising at least a portion of a protein produced in a transformed host organism and at least the amino acid sequence of formula (1). Such fusion proteins form a further aspect of the invention. Desirably the fusion protein includes a protein produced at a high level by a transformed host organism. Suitable such proteins include at least a portion of a chloramphenicol acetyltransferase (CAT) protein or, preferably at least a portion of the β-galactosidase protein.

According to a still further aspect of the invention we provide a DNA sequence coding for a fungal stress protein having substantially the nucleotide sequence of formula (2):

```
          GAATTCAGAGCTATCTTGTTTGTTCCAAAGAGAGCTCCATTTGATGCCTTTGAATCCAAG
                10        20        30        40        50        60
          AAGAAGAAGAACAACATCAAATTATACGTCCGTAGAGTGTTTATCACTGATGATGCTGAA
                70        80        90       100       110       120
          GAGTTGATTCCAGAATGGTTAAGTTTCATCAAGGGGGTTGTCGATTCCGAAGACTTGCCA
    (2)         130       140       150       160       170       180
          TTGAACTTGTCCAGAGAAATGTTGCAACAAAACAAGATTTTGAAAGTTATCAGAAAGAAC
                190       200       210       220       230       240
          ATTGTCAAAAAGATGATTGAAACTTTCAATGAAATCTCTGAAGACCAAGAGCAATTCAAC
                250       260       270       280       290       300
          CAATTCTACACTGCTTTCTCCAAGAACAYCAAAYYHHHYAYYCAYHAAHAYHCYCAAAAC
                310       320       330       340       350       360
          AGACAATCTTTGGCTAAATTGTTGAGATTCTACTCTACCAAATCTTCTGAAGAAATGACT
                370       380       390       400       410       420
          TCCTTGTCTGACTACGTTACTAGAATGCCAGAACACCAAAAGAATATCTACTACATCACT
                430       440       450       460       470       480
          GGTGAATCCATCAAAGCCGTTGAAAAATCACCATTCTTGGATGCCTTGAAAGCTAAGAAC
                490       500       510       520       530       540
          TTTGAAGTCTTGTTCATGGTGGATCCAATCGATGAATATGCCATGACTCAATTGAAGGAA
                550       560       570       580       590       600
          TTTGAAGACAAGAAATTGGTTGATATTACCAAAGACTTTGAATTGGAAGAAAGTGACGAA
                610       620       630       640       650       660
          GAAAAAGCTGCTAGAGAAAAGGAAATCAAAGAATACGAACCATTGACCAAAGCTTTGAAA
                670       680       690       700       710       720
          GATATTCTTGGTGSTCAAGTTGAAAAAGTTGTTGTTTCCTACAAACTTGTTGATGCTCCA
                730       740       750       760       770       780
          GCTGCCATTSGAACTGGTCAATTTGGTTGGTCTGCCAATATGGAAAGAATCATGAAGGCT
                790       800       810       820       830       840
          CAAGCTTTGAGAGACACCACCATGTCTTCTTACATGTCCTCTAAGAAGACCTTTGAAATT
                850       860       870       880       890       900
          TCTCCATCTTCCCCAATTATCAAGGAATTCAAGAAGAAAGTTGAAACCGATGGAGCTGAA
                910       920       930       940       950       960
          GACAAGACCGTTAAGGACTTGACCACTTTGTTGTTTGATACTGCATTGTTGACTTCTGGT
                970       980       990      1000      1010      1020
          TTCACCTTGGACGAACCATCCAACTTTGCCCACAGAATTAACAGATTGATTGCCTTGGGA
               1030      1040      1050      1060      1070      1080
          TTGAATATTGACGATGATTCAGAAGAAACTGCTATTGAACCTGAAGCTACTACTACTGCC
               1090      1100      1110      1120      1130      1140
          TCAACTGACGAACCAGCTGGAGAATCTGCTATGGAAGAAGTTGATTAAACACCAGAAGGG
               1150      1160      1170      1180      1190      1200
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and homologues thereof.

DNA with this sequence may be obtained from fungal genomic DNA as described in the Examples hereinafter.

The DNA sequence according to this aspect of the invention may be incorporated in an expression vector using conventional techniques. Thus in a further aspect of the invention we provide an expression vector including substantially a DNA sequence of formula (2) or a homologue thereof.

The vector may be adapted for use in a given host cell by the provision of suitable selectable markers, promoters and other control regions as appropriate. Host cells transformed with such vectors form a further aspect of the invention. Suitable host organisms include bacteria (e.g. E.coli), and mammalian cells in tissue culture.

The DNA sequence of formula (2) may also be used to design DNA probes for use in identifying the presence of fungal stress proteins in the infected state and the invention xtends to such DNA probes. Such probes may also be of use for detecting circulating fungal nucleic acids, for example using a polymerase chain reaction, as a method of diagnosing fungal infections. The probe may be synthesised using conventional techniques and may be immobilised on a solid phase, capable f immobilisation on a solid phase, or may be labelled with a detectable label.

It will also be appreciated that by suitable epitope mapping, using conventional procedures, for example as described in Example 2 hereinafter peptide fragments of the stress proteins may be identified which can be chemically synthesised. Synthetic peptide antigens of this type may be used to raise antibodies for use in diagnosis and/or therapy, as previously described, or to produce antiseras, .g. non-specific polyclonal antisera, for use as a vaccine, and as discussed above form a further aspect of the invention.

Brief Description of the Drawings

In the following description various embodiments of the present invention are described with reference to the accompanying drawings in which:

Figure 1 - shows immunoblots of C.albicans probed with antigen -selected antibodies.

Figure 2A - shows immunoblots of recombinant lysogen prepared from clone CA-1

Figure 2B - shows immunoblots of recombinant lysogen CA-1 probed with monoclonal antibody against β-galactosidase

Figure 2C - shows immunoblots of recombinant lysogen CA-1 probed with sera from patients having antibody to the 47Kd antigen

Figure 3 - is a comparison of the predicted amino acid sequence of the C.albicans open reading frame (CA-orf) with S.cerevisae hsp90

Figure 4 - shows immunoblots of C.albicans in the yeast phase probed with rabbit antiserum raised against LKVJRKNIVKKMIE-Cys-KLH

Figure 5 - shows immunoblots of C.albicans in the mycelial phase probed with rabbit antiserum raised against LKVIRKNIVKKMIE-Cys-KLH

Description of Specific Embodiments

The following Examples illustrate the invention, in which Example 1 describes the preparation of a stress protein according to the invention; Example 2 describes the epitope mapping of a stress protein according to the invention, the preparation of particular peptide epitopes and their use in detecting Candida infections; Example 3 describes the preparation of a monoclonal antibody against a particular epitope; and Example 4 describes the use of a particular monoclonal antibody [raised against an epitope of a stress protein according to the invention] to protect against Candida infection in mice.

EXAMPLE 1

Strain and culture conditions

A fully characterised strain of C.albicans (serotype A, morphotype A1, biotype 157), responsible for the first London hospital outbreak of systemic candidiasis [Burnie, J.P. et al , Brit. Med. J., 290, 746-748 (1985)] was grown at 37°C overnight with aeration in 2% glucose broth.

Preparation and screening of genomic library

Genomic DNA was prepared by the method of Wills et al [J. Bacteriol. 157 , 918-924 (1984)], as modified by Scherer and Stevens [Proc. Natn. Acad. Sci. USA 85 , 1452-1456, (1988)]. It was partially digested with EcoRI and fragments 2-7 kilobase pairs long were cloned into the unique EcoRI restriction enzyme site of the expression vector lambda gt 11 [Huynh, T. et al in DNA Cloning Techniques: A Practical Approach, Glover, D. Ed, pp. 49-78, IRL Press, Oxford (1985), and Young, R.A. and Davis, P.W., Proc. Natn. Acad. Sci. USA 80 , 1194-1198, (1983)]. The library was screened with rabbit anti serum raised against soluble candidal antigens produced by fragmenting C.albicans yeast cells at -20°C in an X-press [Matthews, R.C. et al , J. Clin. Microbiol. 25 , 230-237 (1987)-(1)]. Immunoblotting against C.albicans , as previously described [Matthews, R.C. et al , Lancet ii, 1415-1418, (1984)-(2); Matthews, R.C. et al J. Clin. Microbiol. ibid], confirmed that the antiserum contained high titre antibody to many antigens including the 47 KD antig n. Five positive clones were identified by screening approximately 10^5 plaques.

Characterisation of positive clones

The epitope expressed by each of the positive clones was identified by antigen-selection as described by Lyon et al [Proc. Natn. Acad. Sci. USA, 83 , 2989-2993, (1986)]. For this the polyspecific rabbit antiserum was affinity purified against positive recombinant plaques and the bound antibody, eluted with glycine buffer pH 2.8, screened against an immunoblot of C.albicans [Matthews, R.C. et al , (1), (2) ibid]. Lysogens were prepared in E.coli Y1089 as describe by Huynh et al [ibid]. To determine whether expression of the fusion protein was under the control of the lac Z promoter, lysates of the recombinant lysogens were examined, by immunoblotting, after: 1) heat-induction at 45°C followed by growth at 37°C for 60 min. with 10mM Isopropyl β-d-thiogalactoside (IPTG); 2) heat induction following by growth at 30°C without IPTG; and 3) growth at 32°C in the presence of 10mM IPTG.

Immunoblots of recombinant lysogens were examined for reactivity of the fusion protein with: 1) rabbit candidal antiserum (diluted 1:25 in 3% bovine serum albumin in buffered saline); 2) a monoclonal antibody to β-galactosidase, obtained commercially from Promega Biotec, Liverpool (1:5,000 dilution); and 3) sera (1:10) from patients with antibody to the 47 KD antigen, including five patients with AIDS and one patient recovering from systemic candidiasis who seroconverted to the 47 KD antigen; sera from five HIV antibody-positive patients with no evidence of candidiasis were used as controls.

DNA sequencing and analysis

Restriction enzyme mapping inserts from the five positive clones identified an overlapping region, suggesting that the epitopes expressed by each clone were encoded by a single genomic segment. This region from clone CA-1, extending 2 KB from the 5′ termini of the insert, was subcloned into the EcoRI site of pUC19. It was sequenced by the dideoxy chain termination method of Sanger et al [Proc. Natn. Acad. Sci. USA 74 5463-5467 (1977)]. Reading frame analysis revealed a single open reading frame (CA-orf) extending from the EcoRI cloning site. The FASTA programme [Pearson, W.R. and Lipman, D.J., Proc. Natn. Acad. Sci. USA, 85 , 2444-2448, (1988)] was used to compare the predicted polypeptide with the PIR protein database.

RESULTS

All five clones expressed epitopes which cross-reacted with the 47 KD antigen and a 92 KD antigen of C.albicans (Fig. 1). Immunoblots of recombinant lysogens, grown with and without IPTG, demonstrated that expression was under the regulation of the lac promoter (Fig. 2A). The cloned antigen was fused to β-galactosidase. The fusion protein produced by clone CA-1 had an elevated molecular weight, Mr 160 KD, compared to native β-galactosidase (Mr 116 KD) (Fig. 2B). A lower Mr band was also seen reacting with the anti-galactosidase monoclonal antibody, indicating the fusion protein was inherently less stable than native β-galactosidase.

As well as the rabbit candidal antiserum, the fusion protein also reacted with sera from AIDS patients with antibody to the 47 KD antigen, but not HIV antibody positive patients without this antibody. A patient with systemic candidiasis who seroconverted to the 47 KD antigen also seroconverted to the fusion protein (Fig. 2C). The patients' sera, which had not been absorbed with E.coli , reacted with several other bands in the recombinant lysogens and the λgt 11 control, and therefore these bands were considered to be E.coli antigens.

Nucleotide analysis of the insert DNA from clone CA-1 revealed a single partial open reading frame (orf) which continued in from the cloning site and coded for a polypeptide of 395 amino acids (Mr 45 KD). This orf was in phase with the β-galactosidase gene. Since fusion with β-galactosidase (Mr 116 KD) results in removal of the C-terminal 19 residues from the lac Z gene, the calculated size of the fusion protein was 159 KD. This agrees with the estimated value of 160 KD for the fusion protein produced by clone CA-1.

A database search, with the polypeptide sequence derived from this orf, revealed significant (>45%) homologies with heat shock proteins (hsps) from Drosophila [Blackman, R.K. and Meselson, M. (1986) J. Mol. Biol. 188, 499-515] and chickens [Kulomaa, M.S., et al (1986) Biochemistry 25 , 6244-6251] and microsomal glucose-regulated proteins (grps) from hamsters [Sorger, P.K. and Pelham, H.R.B., (1987) J. Mol. Biol. 194 , 341-344] and mice [Smith, M.J. and Koch, G.L.E., (1987) J. Mol. Biol. 194 , 345-347]. The most extensive sequence similarity was found with the yeast hsp 90 protein of Saccharomyces cerevisiae [Farrelly, F.W. and Finkelstein, D.B. (1984) J. Biol. Chem. 259 , 5745-5751], with 83.5% identity in the 395

amino acid overlap (Fig. 3).

The following figures are referred to above. In the figures:

Figure 1 shows immunoblots of C.albicans probed with: antigen-selected antibodies from two of th positive clones showing cross-reactivity with the 92 KD and 47 KD antigens, and weakly, two intermediate components (tracks a and b); negative control eluate from non-recombinant plaques (c); rabbit candidal antiserum (d); AIDS patient's serum containing high titre antibody to the 47 KD antigen (e).

Figure 2A shows immunoblots of recombinant lysogen prepared from clone CA-1, probed with rabbit candidal antiserum, showing the 160 KD fusion protein is present when heat induction at 45°C is followed by growth at 37°C for 60 min. with 10mM IPTG (tracks c and d, showing two different lysogenic preparations). It is not produced when lysogens are grown at 32°C (track b) or heat-induced but grown without IPTG (tracks e and f). Molecular weight markers (KD) shown in track a.

Figure 2B shows immunoblots of recombinant lysogen CA-1 probed with monoclonal antibody against β-galactosidase. The 160 KD fusion protein and breakdown product are shown after growth with IPTG (track a), and without IPTG (track b); a non-recombinant λgt11 lysogen, grown with and without IPTG shows the position of native β-galactosidase (Mr 116 KD) (tracks c and d).

Figure 2C shows immunoblots of recombinant lysogen CA-1 probed with sera from patients having antibody to the 47 KD antigen. Serum from an AIDS patient with antibody to the 47 KD antigen cross-reacting with 160KD fusion protein (track a); and early (b) and late (c) sera from a patient recovering from systemic candidiasis who seroconverted to the 47 KD antigen, showing seroconvertion to the 160 KD fusion protein.

Figure 3 is a comparison of the predicted amino acid sequence of the C.albicans open reading frame (CA-orf) with S.cerevisae hsp90 (SCHS90). Over the CA-orf sequence they showed >83% direct homology (:) or >98% conserved homology (.).

EXAMPLE 2

The sequenced carboxy end of a C.albicans stress protein according to the invention was epitope mapped using the method described by Geysen H.M. et al in Journal of Immunological Methods 102 , 259-274 (1987). This technique involves the synthesis of large numbers of overlapping peptides and identifies continuous antigenic peptides on a protein antigen. It will not detect carbohydrate or discontinuous epitopes. By way of example further details of two of these epitopes are given below:

1. STDEPAGESA

This epitope occurs just before the carboxy terminal 5 amino acid residues of the sequence of formula (1). It reacted with: (1) hyperimmune sera from 2 out of 3 rabbits immunised with C.albicans pressate [as described in Burnie J.P., et al J. Clin. Pathol. 38 , 701-706 (1985)]; (2) sera from 3 out of 5 patients with systemic candidiasis, two of whom seroconverted to this epitope; (3) pooled sera from 4 patients who were HIV antibody positive [who have antibody to the 47Kd antigen - see Matthews R.C., et al Lancet ii; 263-266 (1988)]. It did not react with sera from 7 normal control patients or a patient with chronic mucocutaneous candidiasis (CMC).

This epitope was synthesised and conjugated to keyhole limpet haemocyanin (KLH) via the terminal cysteine to give KLH-Cys-STDEPAGESA. A rabbit polyclonal antiserum raised against this, on immunoblotting against C.albicans , recognised a heat-inducible antigen at about 92Kd (present AT 37°C but not 23°C) and a constitutive antigen at about 40Kd (present when grown at 23°C or 37°C). It did not detect the 47Kd antigen. It did not cross-react with S-cerevisiae or A.fumigatus .

2. LSREM-LKVIRK

These two epitopes are situated close to each other, 316-332 amino acid residues from the carboxy end of the sequence of formula (1). The LSREM epitope reacted with; (1) 2 out of 3 rabbit hyperimmune candidal antisera; (2) 4 out of 5 sera from patients with systemic candidiasis, one of whom seroconverted to it; (3) pooled sera from 4 HIV antibody positive patients and (4) serum from the CMC patient. It did not react with sera from 7 negative control patients. A rabbit immunised with KLH-Cys-LPLNLSREML failed to produce antibody to it.

The LKVIRK epitope was specifically detected by infected patients' sera. None of the hyperimmune rabbit sera recognised it nor any of the 7 negativ control patients. All five patients with systemic candidiasis had antibody to this epitope and in two case, where serial sera were available, seroconverted to it. The pooled sera from 4 HIV antibody positive patients and the patient with CMC also recognised this epitope. A rabbit polyclonal antiserum raised against the epitope (LKVIRKNIVKKMIE-Cys-KLH) recognised both the 47 Kd antigen and the antigen of about 92Kd on immunoblots of various strains of C.albicans (including serotypes A and B) in both the yeast and mycelial phase (Figs. 4 and 5). It also recognised the fusion protein produced by a postive clone. Cross-absorption with the synthesised peptide epitope removed this antibody activity. The antibody also recognised antigens on immunoblots of (1) Candida parapsilosis - giving a band at about 52Kd (an antigen of this size was previously reported by Belder M.A., et al European heart Journal 10 , 858-862 [1989]); (2) A.fumigatus - giving bands at about 88Kd, 51Kd and 40Kd [these antigens have previously been reported, Matthews R.C., et al J. Clin. Pathol. 38 , 1300-1303 (1985)] and (3) S.cerevisiae - at about 84Kd and 45Kd; compatible with S.cerevisiae hsp 90. This therefore suggests the presence of a stress protein according to the invention in A. fumigatus of about 88Kd.

In figures 4 and 5 referred to above, the following is shown:

Figure 4 shows immunoblots of C.albicans in the yeast phase probed with rabbit antiserum raised against LKVIRKNIVKKMIE-Cys-KLH both before (PRE) and after (POST) cross-absorption with this peptide. Molecular weight markers shown on left hand side. Lane number 1, outbreak strain of C.albicans [Burnie J.P., et al B.M.J. 290 , 746-748 (1985)]; lane no. 2, C.albicans strain NCTC3153 (serotype A); lane no. 3, C.albicans strain NCTC3156 (serotype B). The 47Kd antigen and the antigen at about 92Kd are arrowed. Notice these antigens are missing after cross-absorption.

Figure 5 Lanes 1-3 are as in figure 5 but with the yeast in the mycelial phase. Probed with the same antiserum as in Figure 4, pre and post cross-absorption with the peptide. Lane no. 4 is a positive clone showing the fusion protein disappearing after cross-absorption, as do the 47Kd and 92Kd antigens in lanes no. 1-3.

Antibody raised against the peptide STDEPAGESA or LKVIRKNIVKKMIE was used to examine the sera of patients with systemic candidiasis as follows:

Antigen was detected by dot-blotting serum directly onto nictrocellulose membrane. Briefly, 100µl of antiserum was loaded into each well of a Bio-Dot microfiltration apparatus (Biorad laboratories). This had been previously loaded with a sheet of nitrocellulose membrane prewashed in pH 7.5 Tris buffered saline (TBS). The sample was allowed to drain through under gravity. Each well was loaded with a further 100µl of TBS which drains under gravity control. This is followed by 2 x 100µl TBS drained by vacuum. The membrane was then blocked in 3% BSA in TBS at 4°C overnight. In the morning it was incubated with 30µl of the appropriate rabbit serum in 3% BSA in TBS for 2 hours at room temperature. It was then washed in 20mM Tris, 500mM NaCl, 0.05% Tween 20, pH 7.5 for 30 minutes and incubated with alkaline phosphatase conjugated antirabbit (1:1000) (Sigma) for 1 hour at room temperature. It was washed again and stained for 15 minutes at room temperature in a freshly prepared and filtered mixture of equal volumes of naphthol AS-MX phosphate (Sigma; 0.4mg/ml in distilled water) and fast red TR salt (Sigma; 6mg/ml in 0.2M Tris, pH 8.2). Antibody intensity was compared by eye with that of controls whose density had been measured previously with a Joyce Loebl scanning densitometer. The results were classified as positive or trace according to the criteria of Matthews, R.C. and Burnie, J.P. (J. Clin. Micro., 26 : 459-463 (1988).

Three groups of sera were examined. The Control sera came from patients who had been screened for systemic candidiasis but where there was no clinical or cultural evidence for the infection. The second group was patients who were colonized at clinically significant sites (intravenous lines, wound, faeces, urine and vagina) and where there was no evidence of dissemination. The third group consisted of either suspected or proven cases. In the suspected cases a clinically significant pyrexia resolved on systemic amphotericin B therapy. The patients were neutropenic and although some cultures were positive for Candida albicans this was insufficient to prove the infection. In the proven cases there was either cultural and histological evidence from a deep site at autopsy or two sets of positive blood cultures taken from separate sites on two different occasions during life.

The sera were examined against antibody raised against the peptide STDEPAGESA or the peptide LRVIRKNIVKKMIE. The former detected (trace or positive response) 90.4% of cases. The latter detected 88.2% of proven and all the suspected cases. There was also a positiv response in four patients where a localised infection required treatment with systemic amphotericin B. Both antibodies detected circulating candidal antigen specific to disseminated candidiasis.

Results of Dot-Blotting			
1. Antibody against STDEPAGESA			
	Nil	Trace	Positive
Controls	65		
Colonized	6	1	
Systemically infected (proven)	2	8	11
2. Antibody against LKVIRKNIVKKMIE			
	Nil	Trace	Positive
Controls	404		
Colonized	10	5	4[a]
Systemically infected			
(proven)	6	9	36
(suspected)		4	6

[a] required systemic therapy for N line infection (1), urinary tract infection (2) and wound infection (1) with amphotericin B.

EXAMPLE 3

A murine monoclonal antibody was raised against LKVIRKNIVKKMIE-Cys-KLH (see Example 2). Balb/c and CBA x Balb/c F1 mice were injected subcutaneously with 50µg of immunogen in sterile complete Freund's Adjuvant and thereafter at intervals of 14 days, intraperitoneally with 50µg immunogen in Incomplete Freund's Adjuvant until seroconvertion.

Fusion was performed 4 days after a final immunisation of 50µg immunogen intravenously in sterile physiological saline. Fusion, hybridoma screening, clonal selection and antibody analysis were performed according to standard protocols, essentially as described by de St. Groth S.F. and Scheidegger D., J. Immunol. Methods 35 , 1-21 (1980). Selected hybridomas were screened for activity against the C.albicans 47Kd antigen by immunoblotting against C.albicans . Positive hybridomas were re-cloned and re-assayed.

A novel hybridoma cell line (CA-STR7-1) was produced which recognised both the C.albicans 47Kd antigen and the antigen of approximately 92Kd on immunoblots of C.albicans grown at 37°C, in both the yeast and mycelial forms. At 23°C the 47Kd antigen was visible but not the 92Kd antigen with this monoclonal antibody.

EXAMPLE 4

A monoclonal antibody was raised against the peptide STDEPAGESA using the method of Example 3, and was used in a mouse model to determine whether it had a protective effect against challenge with a lethal dose of C.albicans . The mouse model was as follows:

Male Balb C mice of average weight 2.5g were injected i.v. with dilutions of a standardised batch of frozen C.albicans . The injected volume was 100µl via the lateral tail vein. The following mortality was observed at the doses and times shown:

	Mortality at		
Dose	18hrs	24hrs	n
5×10^8 cells	89%	100%	54
1×10^8 cells	76%	100%	48
5×10^7 cells	0%	0%	24

The viability of injected cells from frozen aliquats was 30% and therefore the dose could be adjusted downwards.

A challenge dose of 1 x 10^8 C.albicans cells was used as described above in mice which had been pre-treated with the antibody against STDEPAGESA by prior injection as follows:

		Mortality at		"Protective" Agent
	n	24hrs	48 hrs	injected volume
STDEPAGESA Mab	6	66%	83.3%	0.2ml 1hr prior to challenge
Mab L2	6	100%	-	0.2ml 1hr prior to challenge
Patient 1	6	50%	50%	0.5ml 30min prior to challenge
Patient 2	7	42.8%	42.8%	0.5ml 30min prior to challenge
	5	40%	40%	0.5ml 30min prior to challenge
Normal Human Serum	6	100%	-	0.5ml 30min prior to challenge
IG fraction Patient 2	6	50%	66%	0.5ml 30min prior to challenge
IG fraction normal human serum	6	100%	-	0.5ml 30min prior to challenge

"Patient 1" and "Patient 2" was serum from patients who developed systemic Canidida infections, developed an antibody response and recovered.

"IG fraction" was an immunoglobulin fraction obtained by 50% ammonium sulphate precipitation, dialysed overnight v PBS and resuspended in the same volume as the original serum.

Mab L2 was an irrelevant IgG antibody.

Conclusion

The monoclonal antibody raised against STDEPAGESA produced 33% survival at 24 hrs in animals challenged with a lethal dose of C.albicans , whereas the irrelevant IgG and normal human serum produced no protection. The serum from the previously infected patients produced 55% protection at 24hrs.

Claims

1. A fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1):

```
                10        20        30        40        50        60
          EFRAILFVPKRAPFDAFESKKKKNNIKLYVRRVFITDDAEELIPEWLSFIKGVVDSEDLP
5

                70        80        90       100       110       120
          LNLSREMLQQNKILKVIRKNIVKKMIETFNEISEDQEQFNQFYTAFSKNIKLGIHEDAQN
10

     (1)       130       140       150       160       170       180
          RQSLAKLLRFYSTKSSEEMTSLSDYVTRMPEHQKNIYYITGESIKAVEKSPFLDALKAKN
15

                190       200       210       220       230       240
          FEVLFMVDPIDEYAMTQLKEFEDKKLVDITKDFELEESDEEKAAREKEIKEYEPLTKALK
20

                250       260       270       280       290       300
          DILGDQVEKVVVSYKLVDAPAAIRTGQFGWSANMERIMKAQALRDTTMSSYMSSKKTFEI
25

                310       320       330       340       350       360
          SPSSPIIKELKKKVETDGAEDKTVKDLTTLLFDTALLTSGFTLDEPSNFAHRINRLIALG
30

                370       380       390
          LNIDDDSEETAVEPEATTTASTDEPAGESAMEEVD*
```

or a fragment thereof, or an analogue thereof.

2. A stress protein according to Claim 1 obtainable from a strain of Candida, Cryptococcus, Histoplasma and related yeasts or Aspergillus and related filamentous fungi.

3. A stress protein according to Claim 1 or 2 which has one or more of the following features:
   (1) it has an isoelectric point (pI) in a range around pI 4 to pI 5;
   (2) it is an immunodominant conserved antigen;
   (3) patients recovering from systemic candidiasis seroconvert to the stress protein;
   (4) patients with acquired immunodeficiency syndrome have antibody to the stress protein;
   (5) it cross-reacts with the 47 kilodalton and approximately 92 kilodalton antigens of Candida albicans using affinity-purified polyclonal monospecific antibody against the 47 kilodalton antigen.

4. A substantially pure stress protein according to any of Claims 1 to 3.

5. A fragment of a stress protein according to any one of Claims 1 to 3.

6. A fragment according to Claim 5 of sequence STDEPAGESA, LSREM, LKVIRK or LKVIRKNIVKKMIE.

7. A stress protein or fragment according to any one of the preceding claims for use in a diagnostic test for fungal infection.

8. A stress protein or fragment according to Claim 7 wherein the diagnostic test is an enzyme-linked immunosorbent assay, a radioimmunoassay or a latex agglutination assay.

9. A stress protein or fragment according to any one of Claims 1 to 6 for use as an immunogen.

10. An inhibitor of a stress protein or fragment according to Claims 1 to 6.

11. An antibody specific for one or more epitopes of a fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1):

```
                10        20        30        40        50        60
EFRAILFVPKRAPFDAFESKKKKNNIKLYVRRVFITDDAEELIPEWLSFIKGVVDSEDLP

                70        80        90       100       110       120
LNLSREMLQQNKILKVIRKNIVKKMIETFNEISEDQEQFNQFYTAFSKNIKLGIHEDAQN

(1)             130       140       150       160       170       180
RQSLAKLLRFYSTKSSEEMTSLSDYVTRMPEHQKNIYYITGESIKAVEKSPFLDALKAKN

                190       200       210       220       230       240
FEVLFMVDPIDEYAMTQLKEFEDKKLVDITKDFELEESDEEKAAREKEIKEYEPLTKALK

                250       260       270       280       290       300
DILGDQVEKVVVSYKLVDAPAAIRTGQFGWSANMERIMKAQALRDTTMSSYMSSKKTFEI

                310       320       330       340       350       360
SPSSPIIKELKKKVETDGAEDKIVKDLTTLLFDTALLTSGFTLDEPSNFAHRINRLIALG

                370       380       390
LNIDDDSEETAVEPEATTTASTDEPAGESAMEEVD*
```

or a fragment thereof or an analogue thereof.

12. The antibody according to Claim 11 which is specific for a fragment of a fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1)

13. The antibody according to Claim 11 which is specific for the epitope STDEPAGESA, LSREM, LKVIRK or LKVIRKNIVKKMIE.

14. The antibody according to Claim 13 which is specific for the epitope STDEPAGESA.

15. The antibody according to any one of Claims 11 to 14 which is a polyclonal antibody.

16. The antibody according to any one of Claims 11 to 14 which is a monoclonal antibody.

17. The antibody according to any one of Claims 11 to 16 which is labelled with a detectable label or conjugated with an effector molecule.

18. A pharmaceutical composition comprising an antibody according to any one of Claims 11 to 17.

19. An antibody according to any one of Claims 11 to 17 for use in the diagnosis or treatment of fungal infections.

20. An antibody according to Claim 19 for use in the diagnosis or treatment of an infection due to a Candida, Cryptococcus, Histoplasma or Aspergillus organism.

21. A DNA sequence coding for a fungal stress protein having substantially the nucleotide sequence of formula (2):

```
GAATTCAGAGCTATCTTGTTTGTTCCAAAGAGAGCTCCATTTGATGCCTTTGAATCCAAG
      10        20        30        40        50        60
AAGAAGAAGAACAACATCAAATTATACGTCCGTAGAGTGTTTATCACTGATGATGCTGAA
      70        80        90       100       110       120
GAGTTGATTCCAGAATGGTTAAGTTTCATCAAGGGGGTTGTCGATTCCGAAGACTTGCCA
(2)  130       140       150       160       170       180
TTGAACTTGTCCAGAGAAATGTTGCAACAAAACAAGATTTTGAAAGTTATCAGAAAGAAC
     190       200       210       220       230       240
ATTGTCAAAAAGATGATTGAAACTTTCAATGAAATCTCTGAAGACCAAGAGCAATTCAAC
     250       260       270       280       290       300
CAATTCTACACTGCTTTCTCCAAGAACAYCAAAYYHHHYAYYCAYHAAHAYHCYCAAAAC
     310       320       330       340       350       360
AGACAATCTTTGGCTAAATTGTTGAGATTCTACTCTACCAAATCTTCTGAAGAAATGACT
     370       380       390       400       410       420
TCCTTGTCTGACTACGTTACTAGAATGCCAGAACACCAAAAGAATATCTACTACATCACT
     430       440       450       460       470       480
GGTGAATCCATCAAAGCCGTTGAAAAATCACCATTCTTGGATGCCTTGAAAGCTAAGAAC
     490       500       510       520       530       540
TTTGAAGTCTTGTTCATGGTGGATCCAATCGATGAATATGCCATGACTCAATTGAAGGAA
     550       560       570       580       590       600
TTTGAAGACAAGAAATTGGTTGATATTACCAAAGACTTTGAATTGGAAGAAAGTGACGAA
     610       620       630       640       650       660
GAAAAAGCTGCTAGAGAAAAGGAAATCAAAGAATACGAACCATTGACCAAAGCTTTGAAA
     670       680       690       700       710       720
GATATTCTTGGTGSTCAAGTTGAAAAAGTTGTTGTTTCCTACAAACTTGTTGATGCTCCA
     730       740       750       760       770       780
GCTGCCATTSGAACTGGTCAATTTGGTTGGTCTGCCAATATGGAAAGAATCATGAAGGCT
     790       800       810       820       830       840
CAAGCTTTGAGAGACACCACCATGTCTTCTTACATGTCCTCTAAGAAGACCTTTGAAATT
     850       860       870       880       890       900
TCTCCATCTTCCCCAATTATCAAGGAATTCAAGAAGAAAGTTGAAACCGATGGAGCTGAA
     910       920       930       940       950       960
GACAAGACCGTTAAGGACTTGACCACTTTGTTGTTTGATACTGCATTGTTGACTTCTGGT
     970       980       990      1000      1010      1020
TTCACCTTGGACGAACCATCCAACTTTGCCCACAGAATTAACAGATTGATTGCCTTGGGA
    1030      1040      1050      1060      1070      1080
TTGAATATTGACGATGATTCAGAAGAAACTGCTATTGAACCTGAAGCTACTACTACTGCC
    1090      1100      1110      1120      1130      1140
TCAACTGACGAACCAGCTGGAGAATCTGCTATGGAAGAAGTTGATTAAACACCAGAAGGG
    1150      1160      1170      1180      1190      1200
```

and homologues thereof.

22. A DNA sequence according to Claim 21 for use in the design of a DNA probe for the diagnosis of a fungal infection.

23. An expression vector including substantially a DNA sequence according to Claim 21 or a homologue thereof.

24. A process for the production of a fungal stress protein having an amino acid sequence which includes at least the sequence of formula (1) or a fragment thereof or an analogue thereof, comprising the steps of (1) culturing a host organism transformed with a vector including a gene coding for a precursor of said protein, (2) cleaving said precursor to produce said protein and (3) recovering said protein.

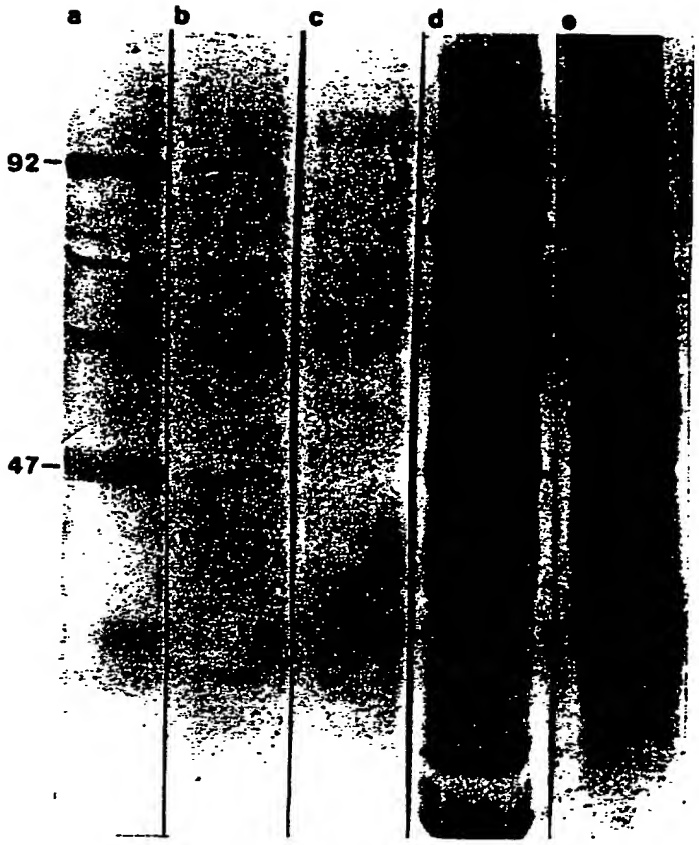

Fig 1

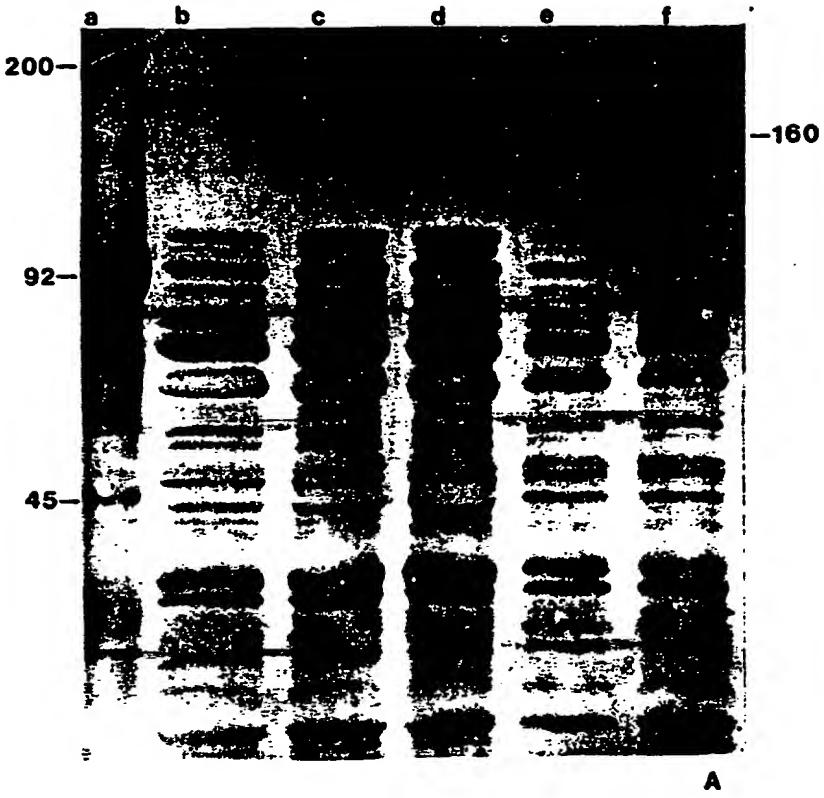

Fig 2A

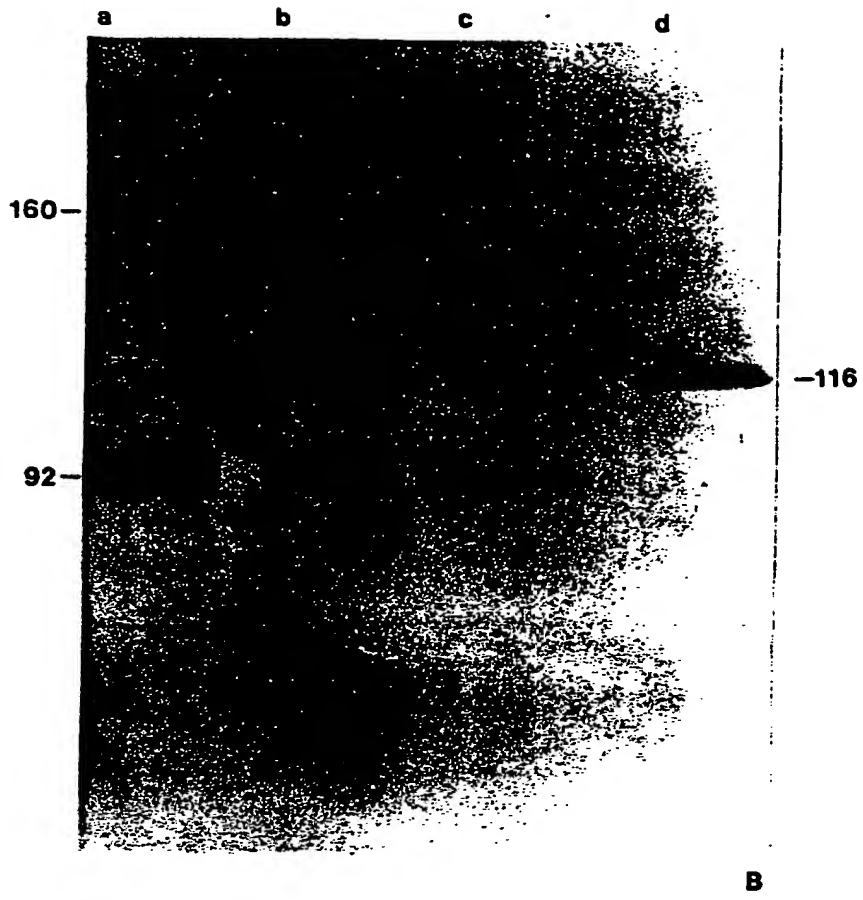

Fig 2B

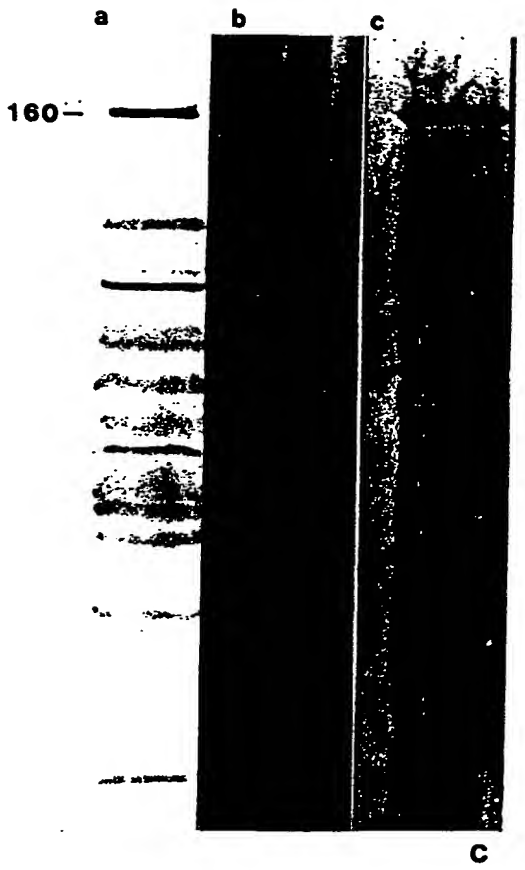

Fig 2C

```
                10        20        30        40        50        60
CA-orf EFRAILFVPKRAPFDAFESKKKKNNIKLYVRRVFITDDAEELIPEWLSFIKGVVDSEDLP
       :::::::.:::::::: ::::::::::::::::::::::::.::.::::::::.::::::::::
SCHS90 EFRAILFIPKRAPFDLFESKKKKNNIKLYVRRVFITDEAEDLIPEWLSFVKGVVDSEDLP
         320       330       340       350       360       370

                70        80        90       100       110       120
CA-orf LNLSREMLQQNKILKVIRKNIVKKMIETFNEISEDQEQFNQFYTAFSKNIKLGIHEDAQN
       :::::::::::::.:::::::::.::.::::.:: ::::..::.:::::::::.:::.::
SCHS90 LNLSREMLQQNKIMKVIRKNIVKKLIEAFNEIAEDSEQFEKFYSAFSKNIKLGVHEDTQN
         380       390       400       410       420       430

               130       140       150       160       170       180
CA-orf RQSLAKLLRFYSTKSSEEMTSLSDYVTRMPEHQKNIYYITGESIKAVEKSPFLDALKAKN
       :..:::::::. :::: .:.:::.:::::::::::::::::::::.::::::::::::::::
SCHS90 RAALAKLLRYNSTKSVDELTSLTDYVTRMPEHQKNIYYITGESLKAVEKSPFLDALKAKN
         440       450       460       470       480       490

               190       200       210       220       230       240
CA-orf FEVLFMVDPIDEYAMTQLKEFEDKKLVDITKDFELEESDEEKAAREKEIKEYEPLTKALK
       :::::..:::::::.:::::::.:.:::::::::::::.:::::.:::::::::::::::::
SCHS90 FEVLFLTDPIDEYAFTQLKEFEGKTLVDITKDFELEETDEEKAEREKEIKEYEPLTKALK
         500       510       520       530       540       550

               250       260       270       280       290       300
CA-orf DILGDQVEKVVVSYKLVDAPAAIRTGQFGWSANMERIMKAQALRDTTMSSYMSSKKTFEI
       .:::::::::::::::.::::::::::::::::::::::::::..:::::::::::::::::
SCHS90 EILGDQVEKVVVSYKLLDAPAAIRTGQFGWSANMERIMKAQALRDSSMSSYMSSKKTFEI
         560       570       580       590       600       610

               310       320       330       340       350       360
CA-orf SPSSPIIKELKKKVETDGAEDKTVKDLTTLLFDTALLTSGFTLDEPSNFAHRINRLIALG
       ::.::::::::.:...::.:::::::.::..:::::::::.:::::..:: :::::::.::
SCHS90 SPKSPIIKELKKRVDEGGAQDKTVKDLTKLLYETALLTSGFSLDEPTSFASRINRLISLG
         620       630       640       650       660       670

               370       380       390
CA-orf LNIDDDSEETAVEPEATTTASTDEPAGESAMEEVD*
       :::::.: :::...:::.:.:...: .....:::::
SCHS90 LNIDED-EETETAPEASTAAPVEEVPADTEMEEVD*
         680       690       700
```

Fig 3

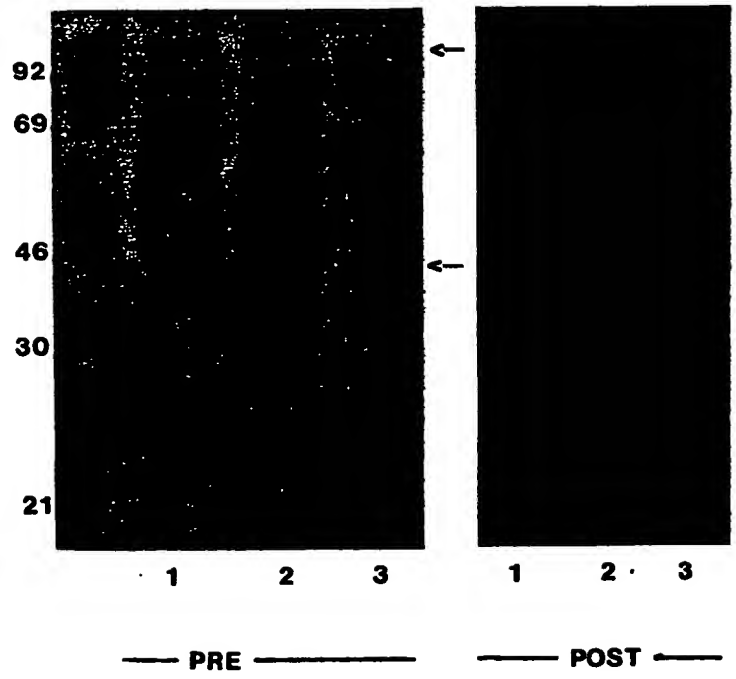

Fig. 4

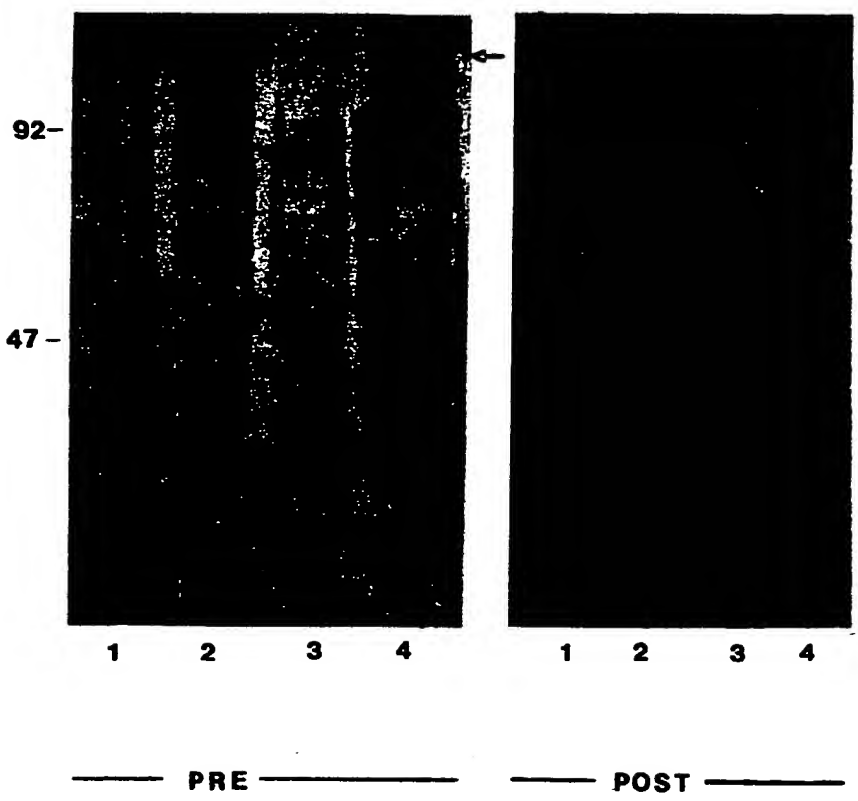

Fig. 5

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EUROPEAN SEARCH REPORT

Application Number

EP 90 30 7236

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	FEMS MICROBIOLOGY LETTERS, vol. 60, 26th June 1989, pages 25-30, Elsevier, Amsterdam, NL; R. MATTHEWS et al.: "Cloning of a DNA sequence encoding a major fragment of the 47 kilodalton stress protein homologue of Candida albicans" * The whole document * ---	1-5,7-9 ,23-23	C 12 N 15/31 A 61 K 39/00 C 12 P 21/08 G 01 N 33/577 G 01 N 33/569 C 07 K 15/00 C 12 N 1/20 // (C 12 N 1/20 C 12 R 1:725)
Y	THE LANCET, no. 8438, 18th May 1985, page 1155, London, GB; J.P. BURNIE et al.: "47kD antigen of Candida albicans" * The whole document * ---	1-4,9, 11-12, 19-20, 21,23	
D,Y	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 259, no. 9, 10th May 1984, pages 5745-5751, The America Society of Biological Chemists, Inc., US; F.W. FARRELLY et al.: "Complete sequence of the heat shock-inducible HSP90 gene of Saccharomyces cervisiae" * The whole document * ---	1-4,7,9 ,11-12, 15-20, 21,23	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
Y	WO-A-8 605 400 (AXON HEALTHCARE LTD, GB) * The whole document * --- -/-	1-4,7,9 ,11-12, 15-20, 23	C 12 N C 12 P A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15-10-1990	LE CORNEC N.D.R.

CATEGORY OF CITED DOCUMENTS

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Application Number

EP 90 30 7236

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,A	JOURNAL OF CLINICAL MICROBIOLOGY, vol. 25, no. 2, February 1987, pages 230-237, American Society for Microbiology; R.C. MATTHEWS et al.: "Isolation of immunodominant antigens from sera of patients with systemic candidiasis and characterization of serological response to Candida albicans" * The whole document * ---	1-4,15, 7-9,21, 23	
A	EP-A-0 145 333 (TEMPLE UNIVERSITY OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION) * The whole document * ---	15-16, 19-20	
A	JOURNAL OF MEDICAL MICROBIOLOGY, vol. 27, 1988, pages 227-232, The Pathological Society of Great Britain and Ireland, London, GB; R. MATTHEWS et al.: "Characterisation and cellular localisation of the immunodominant 47-Kda antigen of Candida albicans" * The whole document * -----	1-3	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

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THE HAGUE	15-10-1990	LE CORNEC N.D.R.

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